94. (new) The method of claim 91, wherein said fragment consists of an amino acid sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46.

95. (new) The method of claim 91, wherein said first amino acid sequence is the amino acid sequence of amino acids 762-1163 of SEQ ID NO:2.

96. (new) The method of claim 82, wherein said fragment is a synthetic peptide.

97. (new) The method of claim 82, wherein said fragment consists of the amino acid sequence of amino acids 623-640 of SEQ ID NO:2.

REMARKS

In the substitute sequence listing, entries under the numerical identifier <213> have been changed to identify the correct species from which the respective sequences are derived. Further, the entries under numerical identifier <221>, <222>, and <223> have been corrected to specify the sites of any unknown nucleotides/amino acids in the respective sequences.

The specification has been amended, so that the SEQ ID Nos refer to the correct sequences.

Claims 11, 16, 30, 52, 53, 55, 58, and 59 have been amended to recite the correct SEQ ID Nos. New claims 63-97 have been added. The new claims are fully supported by the instant specification (see, *e.g.*, the chart below), and do not represent new subject matter.

Claims	Support in Specification
63, 80	page 28, line 1 to page 29, line 32; page 31, line 24-29
64, 65, 73, 82, 84, 92	page 28, line 1 to page 29, line 32; page 25, lines 18-20
67, 74, 85, 93	page 28, line 1 to page 29, line 32; page 59, line 2
66, 68-71, 76, 79, 83, 86-90, 94	page 28, line 1 to page 29, line 32
72, 91	page 28, line 1 to page 29, line 32; page 33, lines 3-7
75, 95	page 28, line 1 to page 29, line 32; page 59, lines 6-10

Claims	Support in Specification
77, 96	page 28, line 1 to page 29, line 32; page 17, lines 4-5
78, 97	page 28, line 1 to page 29, line 32; page 59, lines 4-5
81	page 28, line 1 to page 29, line 32; page 26, lines 16-17

No new matter has been introduced by the above-made amendments. Upon entry of the present amendment, claims 1-97 will be pending in the present application.

Applicants respectfully request entry of the amendments and remarks into the file for the above-identified application.

Respectfully submitted,

Date October 21, 2002

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Enclosures

EXHIBIT A
MARKED-UP VERSION OF THE AMENDED CLAIMS
U.S. PATENT APPLICATION SERIAL NO. 09/830,972

11. (amended) A protein comprising an amino acid sequence that has at least one conservative amino acid substitution in the amino acid sequence depicted in Figure 2a (SEQ ID NO:2), the amino acid sequence depicted in Figure 13 (SEQ ID NO:29) [SEQ ID NO:30] or the amino acid sequence depicted in Figure 14 (SEQ ID NO:32), and which is able to be bound by an antibody directed against a Nogo protein having an amino acid sequence selected from the group consisting of residues 1-1163 of SEQ ID NO: 2, residues 1-172 fused to 975-1163 of SEQ ID NO: 2, and residues 1-199 of SEQ ID NO: 32.

16. (amended) A purified protein comprising a fragment of a Nogo protein comprising an amino acid sequence selected from the group consisting of residues 31-57 depicted in Figure 2a (SEQ ID NO:2), the residues 11-191 depicted in Figure 14 (SEQ ID NO:32), the residues 988-1023 depicted in Figure 2a (SEQ ID NO:2), and residues 1090-1125 depicted in Figure 2a (SEQ ID NO:2), residues 994-1174 depicted in Figure 13 (SEQ ID NO:29 [SEQ ID NO:30]), residues 977-1012 depicted in Figure 13 (SEQ ID NO:29 [SEQ ID NO:30]), and residues 1079-1114 depicted in Figure 13 (SEQ ID NO:29 [SEQ ID NO:30]).

30. (amended) An isolated nucleic acid comprising a nucleotide sequence encoding a protein comprising an amino acid sequence that has a greater than 50% homology to the amino acid sequence of SEQ ID NO:29 [SEQ ID NO:30], as determined by a BLAST computer algorithm.

52. (amended) A purified fragment of a Nogo protein comprising an amino acid sequence selected from the group consisting of amino acid residues 1-131, 132-939, 206-501, 501-680, 132-206, 680-939, and 940-1127 of SEQ ID NO:29 [SEQ ID NO: 30], that is free of all central nervous system myelin material.

53. (amended) A purified fragment of a Nogo protein that lacks amino acid residues 132-206, amino acid residues 939-1127, or amino acid residues 132-206 and 939-1127, of SEQ ID NO:29 [SEQ ID NO: 30] but otherwise comprises the remainder of SEQ ID NO:29 [SEQ ID NO: 30], and that is free of all central nervous system myelin material.

55. (amended) A purified protein comprising a fragment of a Nogo protein, which protein (a) lacks amino acid residues 132-206, amino acid residues 939-1127, or amino acid residues 132-206 and 939-1127, of SEQ ID NO:29 [SEQ ID NO: 30]; and (b) displays the neurite growth inhibitory activity of said Nogo protein, and is free of all central nervous system myelin material.

58. (amended) An isolated nucleic acid that encodes a protein comprising an amino acid sequence selected from the group consisting of amino acid residues 1-131, 132-939, 206-501, 501-680, 132-206, 680-939, and 940-1127 of SEQ ID NO:29 [SEQ ID NO: 30].

59. (amended) An isolated nucleic acid that encodes a protein that lacks amino acid residues 132-206, amino acid residues 939-1127, or amino acid residues 132-206 and 939-1127, of SEQ ID NO:29 [SEQ ID NO: 30] but otherwise comprises the remainder of SEQ ID NO:29 [SEQ ID NO: 30].

EXHIBIT C
MARKED-UP VERSIONS OF AMENDED PARAGRAPHS
(ADDITIONS ARE UNDERLINED, DELETIONS ARE BRACKETED)
U.S. PATENT APPLICATION SERIAL NO. 09/830,972

--Figure 13: The amino acid sequence of rat Nogo A (SEQ ID NO:2) aligned with the theoretical amino acid sequence of human Nogo (SEQ ID NO:29) [(SEQ ID NO:30)]. The human Nogo amino acid sequence was derived from aligning expressed sequence tags (EST) to the rat Nogo sequence and translating the aligned human ESTs using the rat Nogo as a guiding template.--

--Moreover, the present invention provides and includes the predicted amino acid sequence of the human Nogo protein, and fragments thereof. As shown in Figure 13, the amino acid sequence of rat Nogo protein (Figure 2a; SEQ ID NO:2) is aligned with the predicted amino acid sequence of human Nogo protein (Figure 13; SEQ ID NO:29 [SEQ ID NO:30]). Accordingly, the present invention encompasses human Nogo proteins comprising the predicted amino acid sequence of human Nogo, Figure 13 and SEQ ID NO:29 [SEQ ID NO:30], or a subsequence of the predicted amino acid sequence of human Nogo, consisting of at least 6 amino acid residues, or one or more of the following predicted amino acid sequences of human Nogo fragments: MEDLDQSPLVSSS (Human Nogo, corresponding to amino acids 1-13 with SEQ ID NO:43), KIMDLKEQPGNTISAG (Human Nogo, corresponding to amino acids 187-203 with SEQ ID NO:44), KEDEVVSSEKAKDSFNEKR (Human Nogo, corresponding to amino acids 340-358 with SEQ ID NO:45), QESLYPAAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASVIQPSS (Human Nogo, corresponding to amino acids 570-619 with SEQ ID NO:46). Naturally occurring human Nogo and recombinant human Nogo, and fragments thereof having an amino acid sequence substantially similar to the above-described amino acid sequences and able to be bound by an antibody directed against a Nogo protein are within the scope of the invention.--

--The present invention further provides nucleic acid molecules that encodes a human Nogo protein having an amino acid sequence substantially similar to the amino acid sequence as shown in Figure 13 (Figure 13; SEQ ID NO:29 [SEQ ID NO:30]). In specific

embodiments, nucleic acid molecules encoding fragments of human Nogo protein having an amino acid sequence substantially similar to the amino acid sequence as shown in Figure 13 (SEQ ID NO:29 [SEQ ID NO:30]) are also contemplated with the proviso that such nucleic acid molecules do not comprise the nucleotide sequence of the above-identified human ESTs.--

--To perform functional analysis of various regions of Nogo, a series of deletions in the *Nogo* gene has been generated and cloned into an expression vector by recombinant DNA techniques and expressed as a fusion protein. Nucleic acids that encode a fragment of a Nogo protein are provided, e.g., nucleic acids that encode amino acid residues 1-171, 172-974, 259-542, 542-722, 172-259, 722-974, or 975-1162 of SEQ ID NO: 2, or combinations thereof; and nucleic acids that encode amino acid residues 1-131, 132-939, 206-501, 501-680, 132-206, 680-939, and 940-1127 of SEQ ID NO:29 [SEQ ID NO:30], or combinations thereof. Some of the deletion constructs comprises truncated portions of Nogo and additional nucleotide sequences encoding a hexahistidine tag and/or a T7-tag. Nucleic acids encoding truncated Nogo proteins that lacks amino acid residues 172-259, amino acid residues 974-1162, or amino acid residues 172-259 and 974-1162, of SEQ ID NO:2 but otherwise comprises the remainder of SEQ ID NO: 2; or amino acid residues 132-206, amino acid residues 939-1127, or amino acid residues 132-206 and 939-1127, of SEQ ID NO:29 [SEQ ID NO:30] but otherwise comprises the remainder of SEQ ID NO:29 [SEQ ID NO:30], are provided. The structure of exemplary deletion constructs are shown in Figure 18. The deletion constructs produce fragments or truncated portion(s) of Nogo when introduced into a cell. The biological activities of these mutants were tested in various functional assays as described in Table 2 in Section 6.2.7.--

--Nucleotide sequences encoding fragments of human Nogo A comprising an amino acid sequence selected from the group consisting of amino acid residues 1-131, 132-939, 206-501, 501-680, 132-206, 680-939, and 940-1127 of SEQ ID NO:29 [SEQ ID NO:30] are also provided. Nucleotide sequences that encodes truncated portions of human Nogo A are also provided; the truncated proteins lack amino acid residues 132-206, amino acid residues 939-

1127, or amino acid residues 132-206 and 939-1127, of SEQ ID NO:29 [SEQ ID NO:30] but otherwise comprises the remainder of SEQ ID NO:29 [SEQ ID NO:30].--

--In a specific embodiment of the present invention, such Nogo proteins, whether produced by recombinant DNA techniques or by chemical synthetic methods or by purification of native proteins, include but are not limited to those containing, as a primary amino acid sequence, all or part of the amino acid sequence substantially as depicted in Figure 2a (SEQ ID NO:2), bovine in Figure 12 (SEQ ID NO:28 [SEQ ID NO:29]), or human in Figure 13 (SEQ ID NO:29 [SEQ ID NO:30), as well as fragments and other derivatives (such as but not limited to those depicted in Figure 18), and analogs thereof, including proteins homologous thereto. Preferably, the Nogo proteins of the invention are free of all CNS myelin material with which it is normally associated.--

--Various procedures known in the art may be used for the production of polyclonal antibodies to a Nogo protein or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of a Nogo protein encoded by a sequence of SEQ ID NO:2 in Figure 2a, SEQ ID NO:28 [SEQ ID NO:29] in Figure 12, SEQ ID NO:32 in Figure 14, or SEQ ID NO:29 [SEQ ID NO:30] in Figure 13, (rat Nogo A, bovine Nogo, rat Nogo C, or human Nogo respectively) or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native Nogo protein, or a synthetic version, or derivative (*e.g.*, fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.--

--In order to map the active region(s) of Nogo, a series of Nogo deletion mutants have been prepared by recombinant DNA techniques as described in Section 6.2.7. The portions of Nogo which are present in the deletion mutants are shown in Figure 18. In a specific

embodiment, the invention provides fragments of Nogo e.g., fragments comprising (or alternatively consisting of) Nogo A (SEQ ID NO: 2) amino acid numbers 1-171, 172-974, 259-542, 542-722, 722-974, 172-259, or 975-1162, or combinations of the foregoing. Truncated mutants of Nogo lacking amino acid numbers 172-259 and/or 975-1162 of SEQ ID NO:2 are also provided, as these regions appear to be non-essential and can be removed from Nogo without affecting biological activity. The corresponding fragments of human Nogo A comprising (or alternatively consisting of) amino acid numbers 1-131, 132-939, 206-501, 501-680, 132-206, 680-939, or 940-1127 of SEQ ID NO:29 [SEQ ID NO:30] are also provided. Truncated mutants of human Nogo A are also provided which lack amino acid numbers 132-206, amino acid residues 939-1127, or amino acid residues 132-206 and 939-1127, of SEQ ID NO:29 [SEQ ID NO:30].--

--The instant invention provides the nucleotide sequences encoding human Nogo protein, and fragments of human Nogo proteins, including the human equivalents to rat Nogo A, Nogo B and part of Nogo C. The human Nogo amino acid sequence is depicted in Figure 13 and has been assigned SEQ ID NO:29 [SEQ ID NO:30].--